

# **Haematology and blood transfusion**

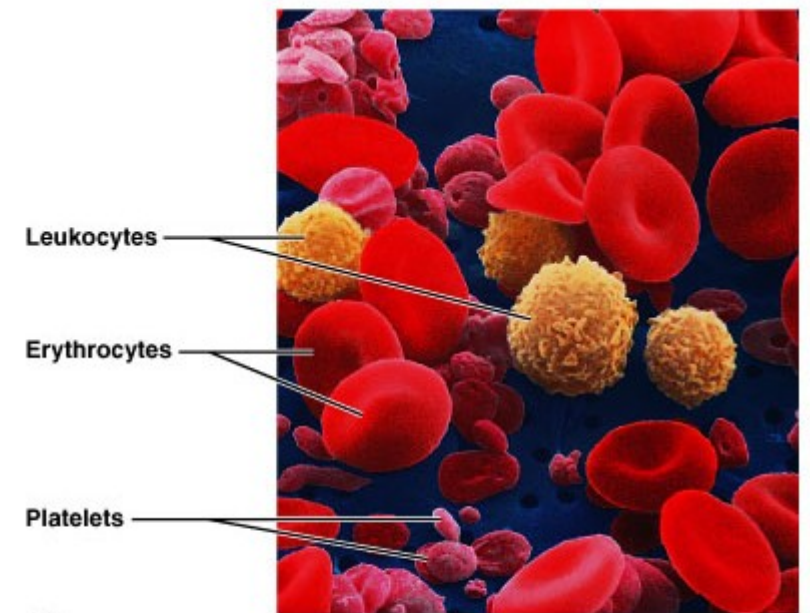
Andrea Knight, PhD

# Definition & function

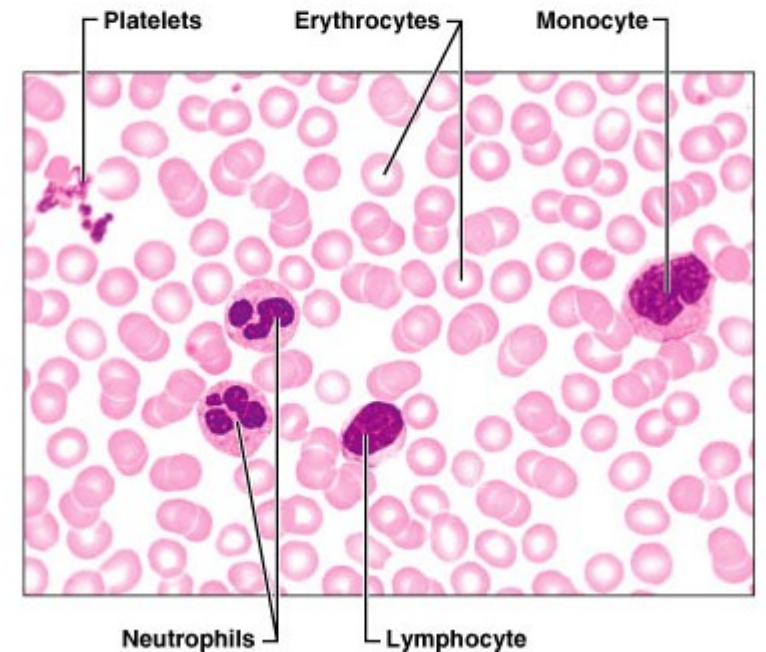
- the branch of medical science concerning blood and blood-forming tissues
- study of etiology, diagnosis, treatment, prognosis & prevention
- pathophysiology
  - Variations from normal blood element counts
  - Bleeding disorders - hemophilia
  - Malignant disorders – leukaemia, lymphoma, myeloma
  - Haemoglobinopathies
- bone marrow and stem cell transplantation
- blood transfusion & blood banking

# Organs & tissues

- peripheral blood
- bone marrow
  
- spleen
- lymph nodes
- (liver)

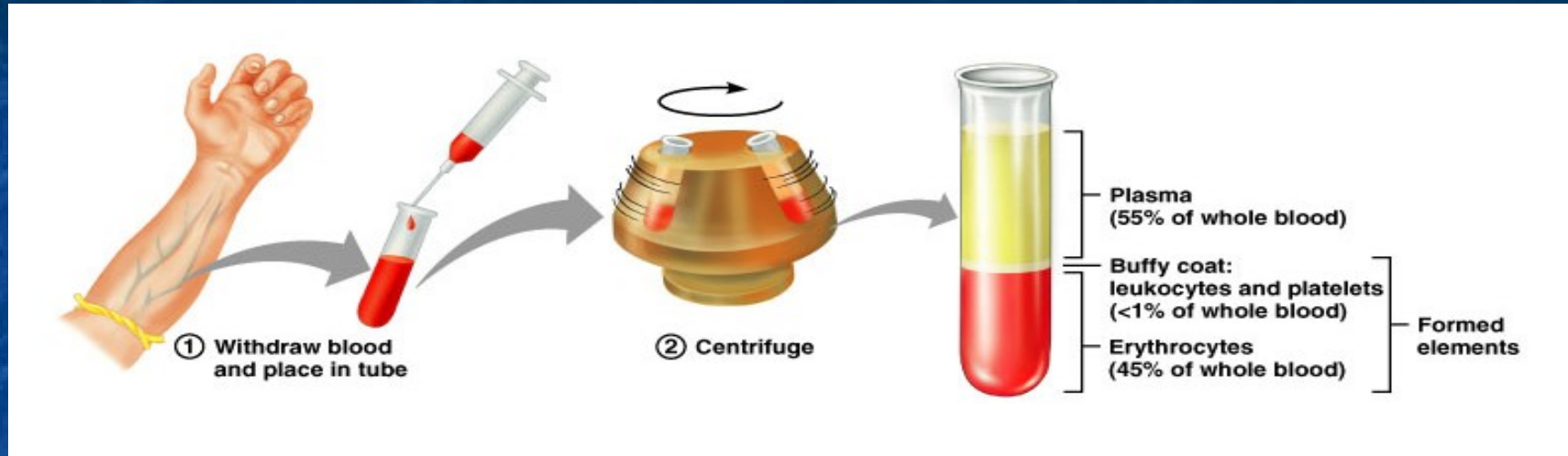


(a)



# Composition of blood

- Specialized connective tissue
- Blood cells (elements) suspended in plasma
- Blood volume: 5-6 litres in males and 4-5 litres in females
- Clinically important hematocrit
  - % of blood volume consisting of erythrocytes (red blood cells)
  - Male average 47; female average 42
  - Plasma contains water, ions, 3x important proteins: albumin, globulins, fibrinogen
- Serum
  - Blood that is allowed to stand will clot
  - plasma without the clotting factors



- Density gradient centrifugation
- Solution: Lymphoprep, Ficoll (1.077 g/ml)
  - layered over with whole blood or bone marrow as 1:1 volumes
- Buffy coat
  
- Practicals: small groups 4-6 students welcome to learn in our labs; to arrange by email: [knight@med.muni.cz](mailto:knight@med.muni.cz)

## Anticoagulants:

-to prevent the [coagulation](#) (clotting) for:

renal dialysis

deep vein thrombosis (DVT)

pulmonary embolism

myocardial infarction

ischemic stroke

-food supplements with blood-thinning effects: beer, papaya, cranberries

-encouraging clotting are avocado, spinach



A key event in the [blood coagulation](#) is the conversion of [fibrinogen](#) into [fibrin](#) by the [serine protease](#) enzyme [thrombin](#).

Thrombin is produced from [prothrombin](#), by the action of an enzyme, prothrombinase (Factor Xa along with Factor Va as a cofactor), in the final states of coagulation.

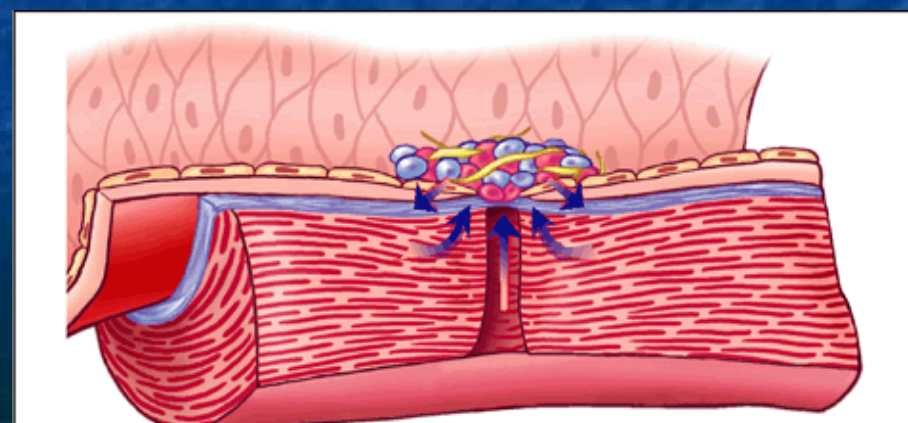
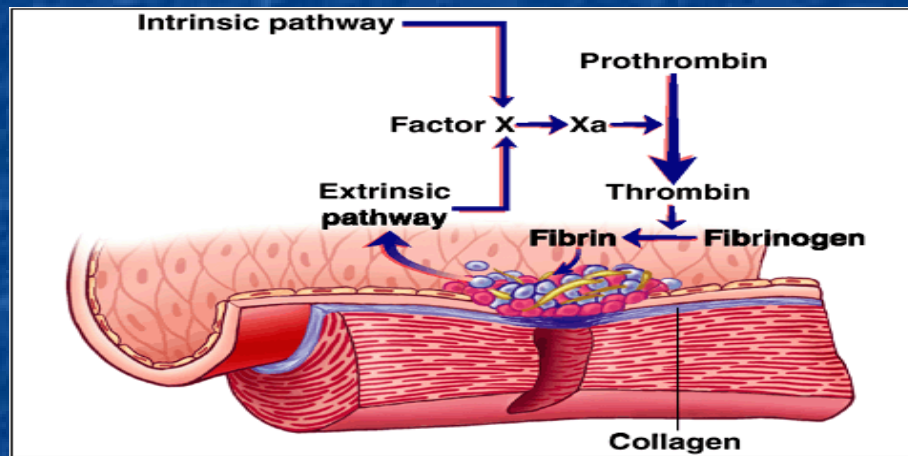
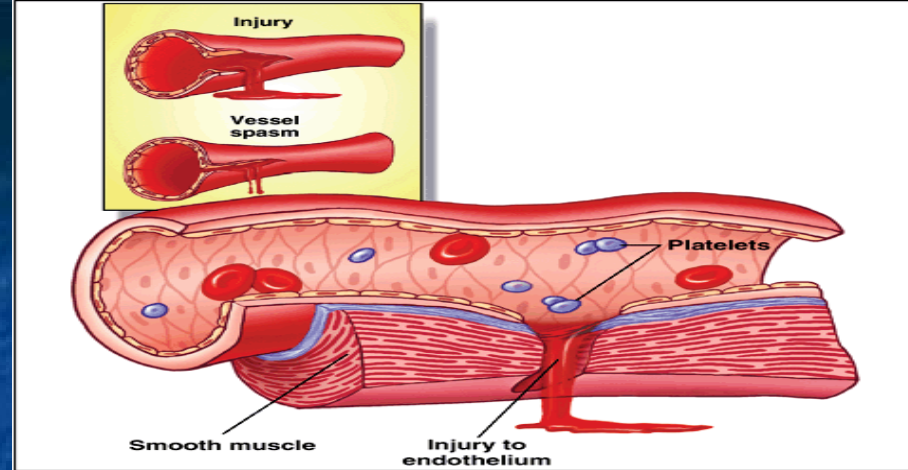
Fibrin is then cross linked by factor XIII (Fibrin Stabilizing Factor) to form a [blood clot](#).

The principal [inhibitor](#) of [thrombin](#) in normal blood circulation is [antithrombin](#).

# Zástava krvácení

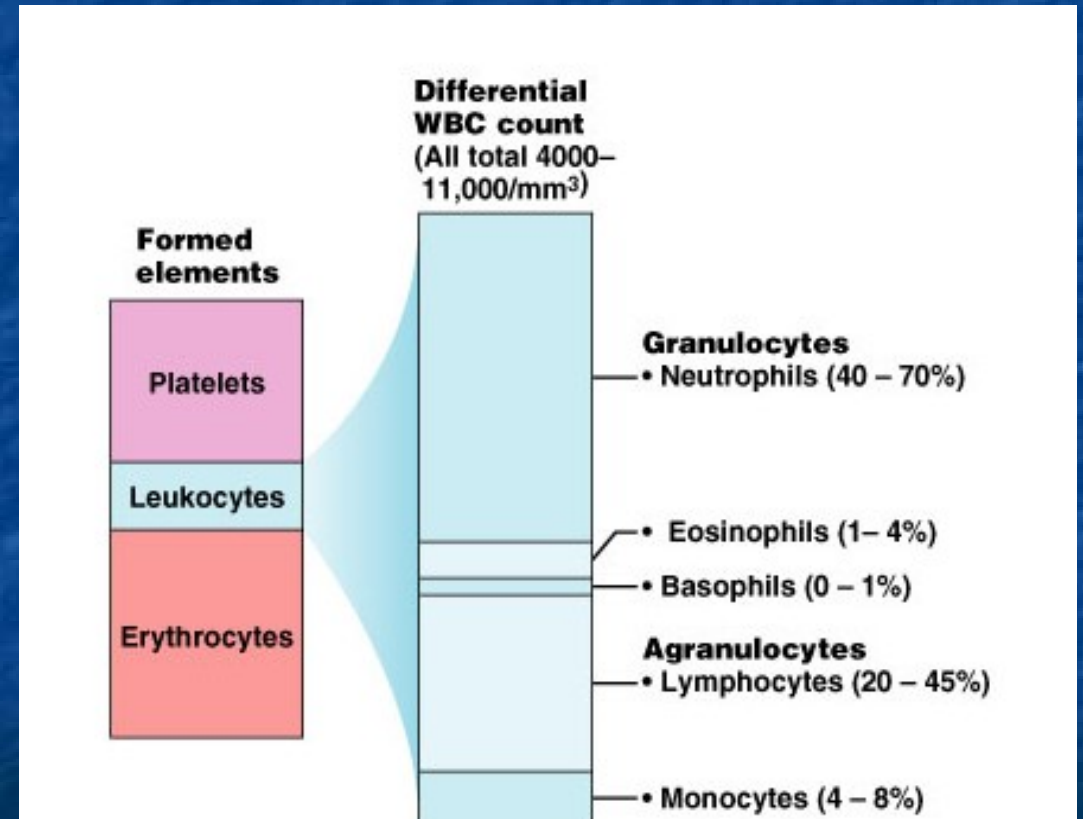
■ hemostáza má tři složky:

1. reakce cév
2. reakce trombocytů
3. koagulace: srážení krve



# CBC test = complete blood count

- RBC count (red blood)
- WBC (white blood)
  - in thousands/cumm
  - differential WBC
- Platelet count in thousands/cumm





# Adult Normal Ranges in FBC

Parameter		Male	Female
<b>Haemoglobin</b>	g/dL	13.5 - 18.0	11.5 - 16.0
<b>WBC</b>	x10 <sup>9</sup> /L	4.00 - 11.00	4.00 - 11.00
<b>Platelets</b>	x10 <sup>9</sup> /L	150 - 400	150 - 400
<b>MCV</b>	fL	78 - 100	78 - 100
<b>PCV</b>		0.40 - 0.52	0.37 - 0.47
<b>RBC</b>	x10 <sup>12</sup> /L	4.5 - 6.5	3.8 - 5.8
<b>MCH</b>	pg	27.0 - 32.0	27.0 - 32.0
<b>MCHC</b>	g/dL	31.0 - 37.0	31.0 - 37.0
<b>RDW</b>		11.5 - 15.0	11.5 - 15.0
<b>Neutrophils</b>		2.0 - 7.5	2.0 - 7.5
<b>Lymphocytes</b>		1.0 - 4.5	1.0 - 4.5
<b>Monocytes</b>		0.2 - 0.8	0.2 - 0.8
<b>Eosinophils</b>		0.04 - 0.40	0.04 - 0.40
<b>Basophils</b>		< 0.1	< 0.1

- MCV: mean cell volume
- PCV: packed cell volume (or haematocrit) – percentage of the blood volume that is made from RBCs
  - Hypoxia can lead to increased amount of RBC
- MCH: mean cell haemoglobin – average amount of haemoglobin per RBC
- MCHC: mean cell Hb concentration
- RDW: red cell distribution width

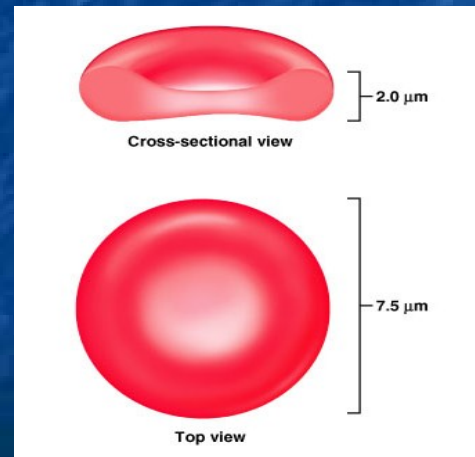
# Variations from normal

- Lymphopenia : **too few** lymphocytes
- Neutropenia: **too few** neutrophils
- Thrombocytopenia : **too few** platelets
  
- Neutrophilia: **too many** neutrophils
- Thrombocytosis: **too many** platelets
- Leucocytosis : **too many** WBC

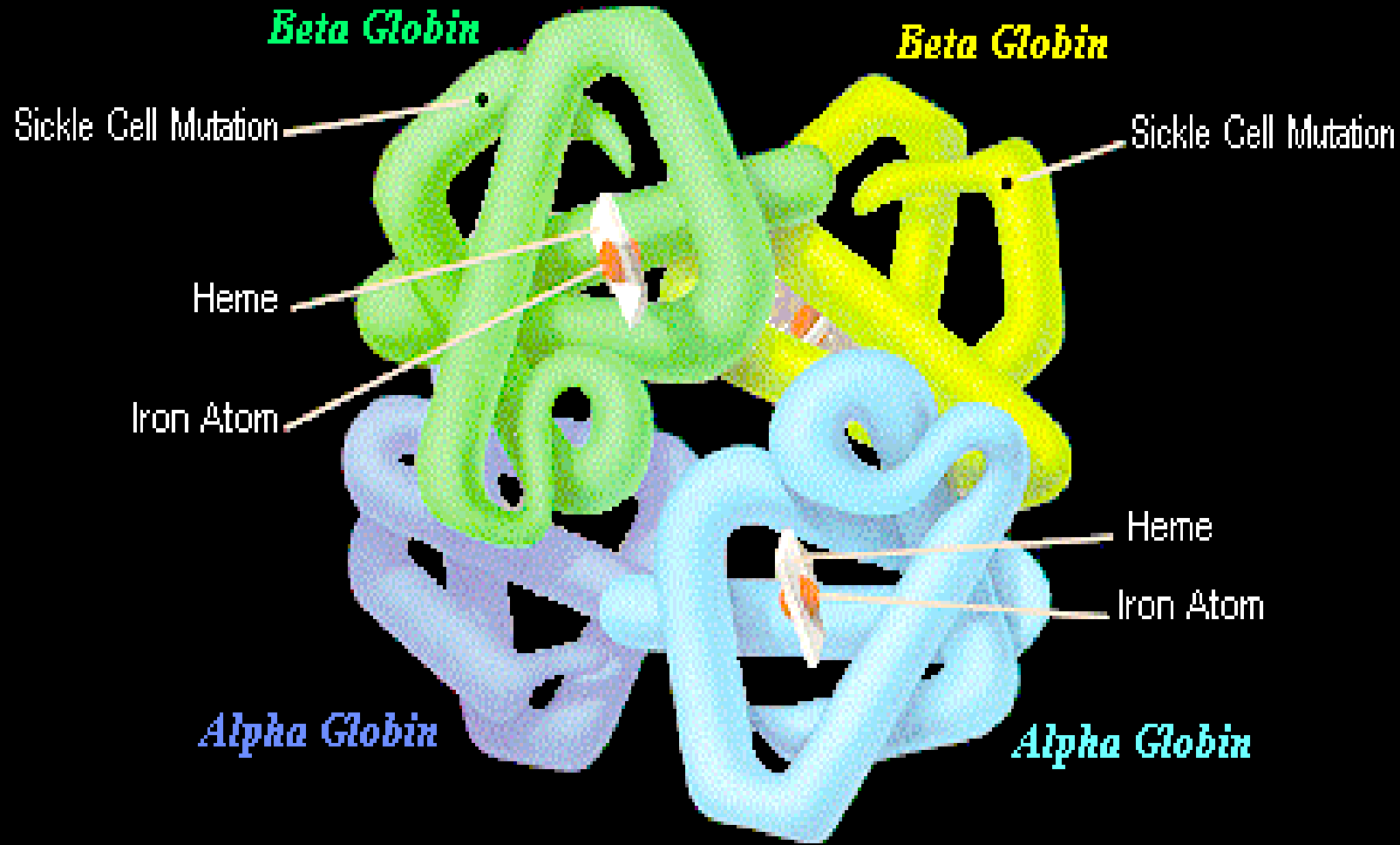
# Erythrocytes

- Also called red blood cells (RBC)
- Biconcave discs and flexible
- Plasma membrane but no nuclei or organelles
- Packed with **hemoglobin** molecules
  - Oxygen carrying protein
  - 4 chains of amino acids, each with iron which is binding site for oxygen/CO<sub>2</sub>
  - young RBC still containing ribosomes are called **reticulocytes**
- Lifespan 100-120 days

Parameter	Male	Female
<b>Haemoglobin</b> g/L	135 - 180	115 - 160
<b>RBC</b> x10 <sup>12</sup> /L	4.5 - 6.5	3.8 - 5.8



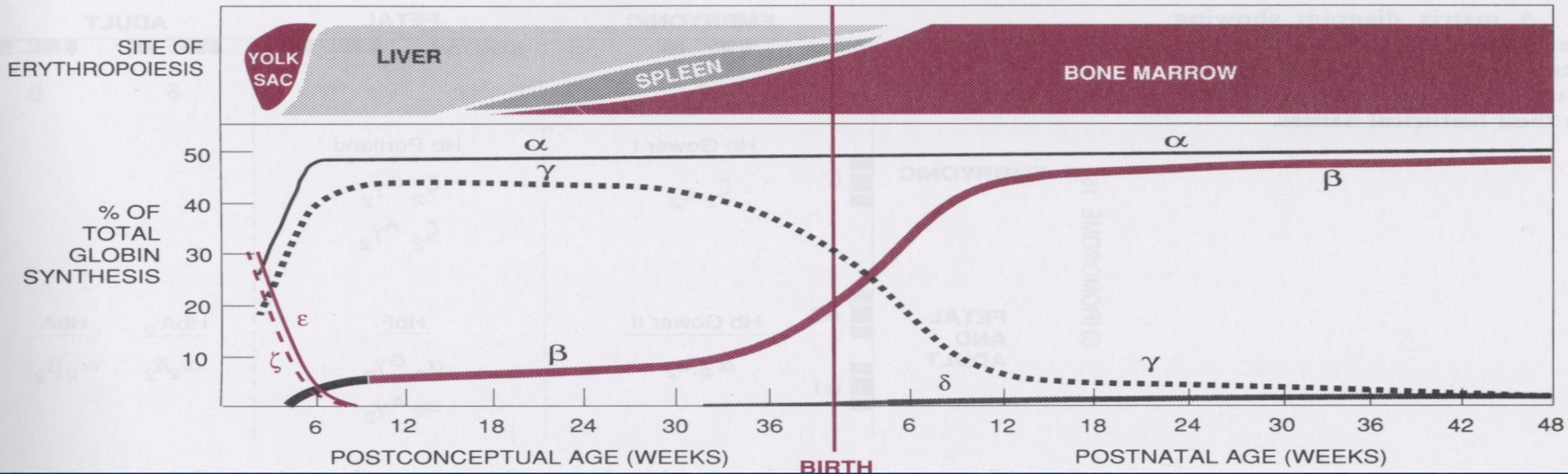
# HEMOGLOBIN



# Hemoglobin

- Heterotetramer
- HbA<sub>1</sub>  $\alpha_2\beta_2$  96-98%
- HbA<sub>2</sub>  $\alpha_2\delta_2$  2%
- HbF  $\alpha_2\gamma_2$  this dominates until 6 weeks of age
- Afterwards, Hb A dominates through life

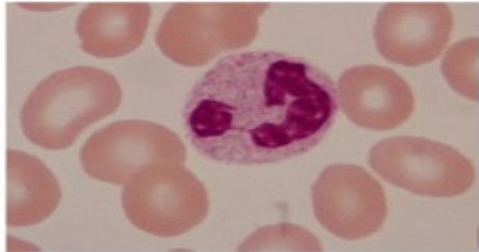
# Erythropoiesis



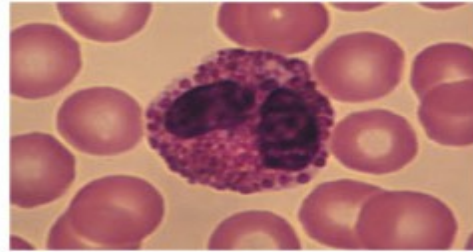
# Methemoglobin

- MetHb is the derivative of Hb, in which the iron of the heme group is oxidized from  $\text{Fe}^{2+}$  to  $\text{Fe}^{3+}$
- MetHb is no longer completely capable of reversibly binding  $\text{O}_2$  (brown)
- MetHb forms continuously (present in RBC 1-2% c HB)
  - must be reduced actively by normal red cell metabolism or by ascorbic acid
  - cyanosis & fatigue 10%, coma & fatal 50-70%
  - nitrates in food and water, medication-local anesthetics, G6PD deficiency

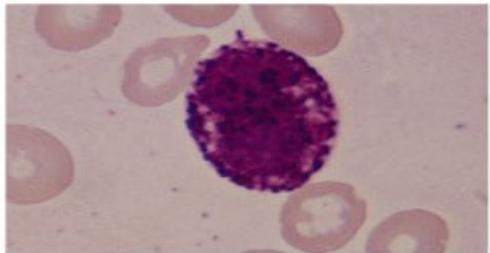
# Leukocytes



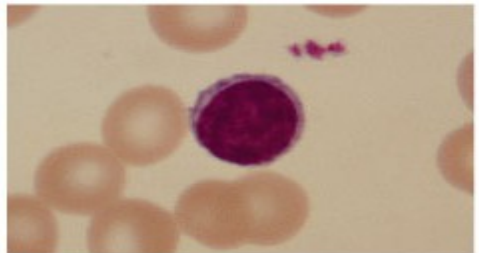
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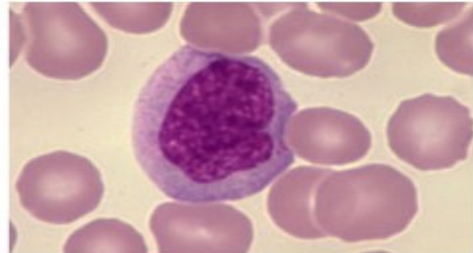
(b)



(c)



(d)



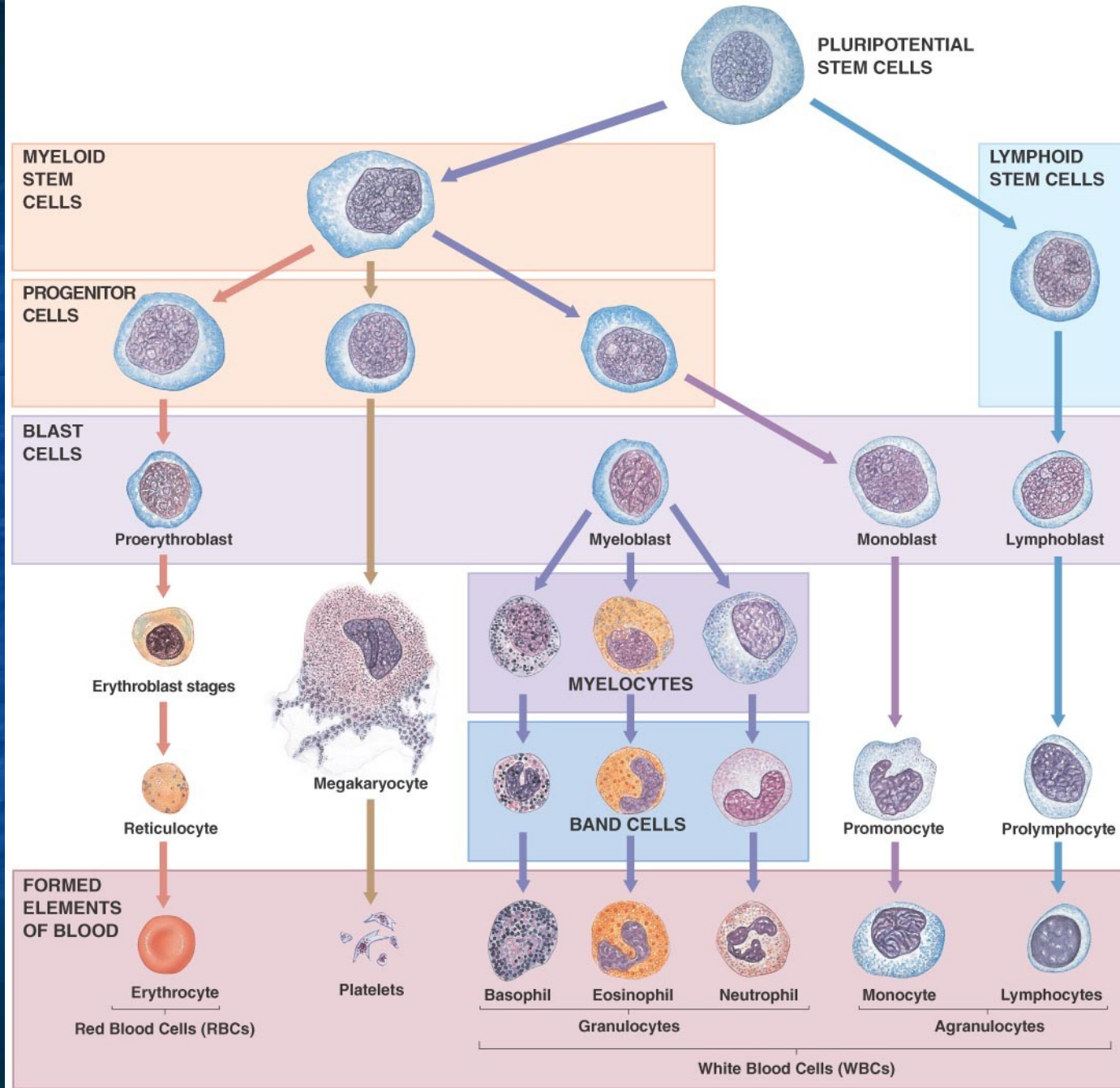
(e)

- A) Granulocytes
  - Granules, lobed nuclei
  - All phagocytic
  - Neutrophil: Nuclei of 2-6 lobes
  - Eosinophil: Nuclei bi lobed
  - Basophil: Dark purple granules
- D) lymphocyte
  - Large nucleus
  - T, B lineage
  - NK
- E) monocyte diff. into MØ



# Hematopoiesis

- Formation of blood cells
- Occurs mostly in red bone marrow
- All cells arise from same pluripotent hematopoietic stem cells
- MSCs form fat cells, osteoblasts, chondrocytes, fibroblasts and muscle cells



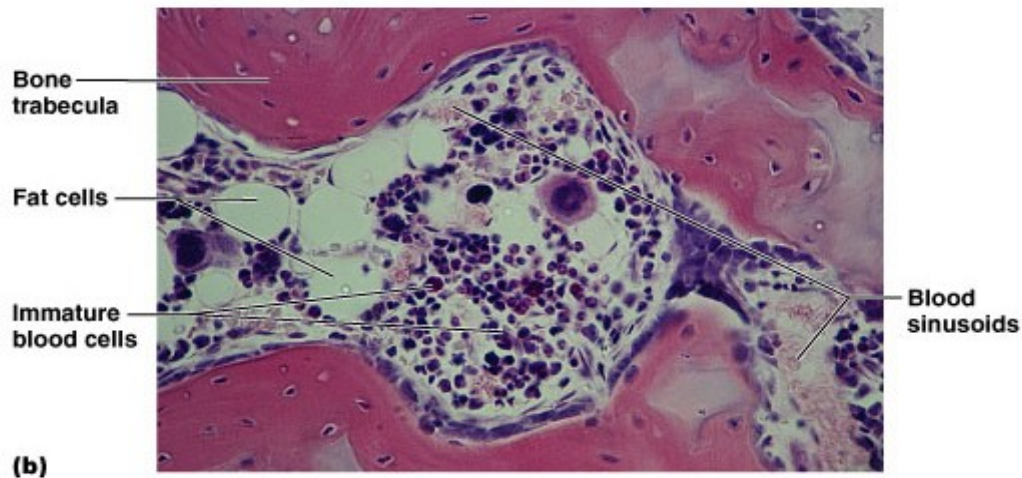
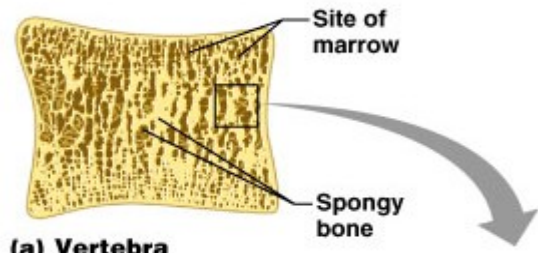
*Not shown are mast cells, osteoclasts, dendritic cells*

# Bone marrow

- Red marrow (medulla ossium rubra)
  - Consists mainly of haematopoietic tissue
  - Site of haematopoiesis (red and white blood cells, platelets)
- Yellow marrow (medulla ossium flava)
  - Made up of fat cells
- With age more red BM is converted to yellow BM
- BM stroma
  - Creates a microenvironment
    - Fibroblasts, MØ, adipocytes
    - Osteoblasts, osteoclasts
    - Endothelial cells
- Mesenchymal Stem cells (MSC)
  - Pluripotent stem cells that can differentiate *in vitro* and *in vivo* into a number of cell types incl. osteoblasts, chondrocytes, myocytes, adipocytes

# Examination of bone marrow

- BM sample obtained via biopsy or aspiration
- Used to newly diagnose & confirm suspected pathology
- To examine haematopoiesis
- Parallel to analysis of PB drawn from a vein by a phlebotomist
- Invasive procedure, not a routine



# Bone marrow harvest for transplantation

- BM is collected (pelvis under general anesthesia) and infused back:
  - Autologous Tx - same patient
  - Allogeneic Tx
    - Matched sibling
    - Matched Unrelated Donor (MUD)
  - Donor – recipient compatibility (MHC/HLA alleles)
  - Donor registers around the world

# Cell storage for transplantation

- Cells frozen in 5-10% DMSO/human serum
  - DMSO, Dimethyl sulfoxid
  - Prevents the formation of ice crystals during the freezing process
- Stored at liquid nitrogen (-196 °C) for months/years
- Decreasing the temperature as 1°C per minute over night at -80°C in the Mr Frostie containing isopropyl alcohol

# The Anthony Nolan Trust

- <http://www.anthonynolan.org/>
  - story of Anthony Nolan (1971-1979)
  - born with a rare Wiskott-Aldrich syndrome
  - only cure was Tx but no donor available
  - Shirley Nolan (1942-2002) and her legacy to start a donor register
  - Currently over 500 000 donors fully typed
  - Important charity – please log-in & donate
  - Research Institute & project Allostem
    - major EU grant involved 13 countries including CZ (Prof. Bartunkova, Prague)
  - Essential contribution to EBMT



# MHC proteins

- Major Histocompatibility Complex, chr. 6
- HLA, human leukocyte antigens
  - Transplant antigens to prevent graft rejection
- HLA I. class (HLA-A, B, C)
  - Expressed on all nucleated cells
- HLA II. class (HLA-DP, DQ, DR)
  - Expressed on cells of IS
- MHC III. class
  - complement
- Prof. S Marsh at ANRI, President of the European Federation for Immunogenetics
- Allele frequencies vary in different populations and ethnic groups

# Haematopoietic stem cell transplantation

- Progenitor stem cell transplantation derived from:
  - BM
  - peripheral blood
  - cord blood
- **Autologous Tx**
  - Requires extraction/apheresis of stem cells (HSC)
  - Stored in the liq nitrogen
  - Patient undergoes high-dose chemo ± radiotherapy
  - Established as the second-line treatment for lymphoma (not for AML)
- **Allogeneic Tx**
  - HLA matching
  - Recipient's immunosuppression
  - full ablative vs Reduced intensity conditioning (RIC)
  - RIC pioneered by **Prof Stephen Mackinnon** at University College London
  - Numerous clinical trials ongoing

# Post HSCT

- Cytokine storm
- **Graft-versus-host disease (GvHD)** as a major complication post SCT
  - T cells present in the transplant recognize the host's (recipient's) cells as foreign
  - Minor histocompatibility antigens
  - **Acute** within 100 days as major challenge to transplant mortality and morbidity (grade 1-4)
  - **Chronic** as moderate to severe
  - Skin, liver, gut and GI tract, lung
  - Donor T cells mediate **graft -versus-tumour effect** (versus leukaemia, lymphoma or myeloma)

# Graft – versus - tumour effect

- GvL (versus leukaemia)
  - Most prominent in CML patients, (also in ALL)
- GvM (myeloma)

*Cytotherapy*, 2012; 14: 1110–1118

**informa**  
healthcare

## **Human Vdelta1 gamma-delta T cells exert potent specific cytotoxicity against primary multiple myeloma cells**

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*Department of Haematology, Royal Free Hospital, University College Medical School London, UK*

### **Abstract**

*Background aims.* Human gamma-delta ( $\gamma\delta$ ) T cells are potent effector lymphocytes of innate immunity involved in anti-tumor immune surveillance. However, the V $\delta$ 1  $\gamma\delta$  T-cell subset targeting multiple myeloma (MM) has not previously been investigated. *Methods.* V $\delta$ 1 T cells were purified from peripheral blood mononuclear cells of healthy donors and patients with MM by immunomagnetic sorting and expanded with phytohemagglutinin (PHA) together with interleukin (IL)-2 in the presence of allogeneic feeders. V $\delta$ 1 T cells were phenotyped by flow cytometry and used in a 4-h flow cytometric cytotoxicity assay. Cytokine release and blocking studies were performed. Primary myeloma cells were purified from MM patients' bone marrow aspirates. *Results.* V $\delta$ 1 T cells expanded from healthy donors displayed prominent cytotoxicity by specific lysis against patients' CD38<sup>+</sup> CD138<sup>+</sup> bone marrow-derived plasma cells. V $\delta$ 1 T cells isolated from MM patients showed equally significant killing of myeloma cells as V $\delta$ 1 T cells from normal donors. V $\delta$ 1 T cells showed similarly potent cytotoxicity against myeloma cell lines U266 and RPMI8226 and plasma cell leukemia ARH77 in a dose-dependent manner. The interferon (IFN)- $\gamma$  secretion and V $\delta$ 1 T-cell cytotoxicity against myeloma cells was mediated in part through the T-cell receptor (TCR) in addition to involvement of Natural killer-G2D molecule (NKG2D), DNAX accessory molecule-1 (DNAM-1), intracellular cell adhesion molecule (ICAM)-1, CD3 and CD2 receptors. In addition, V $\delta$ 1 T cells were shown to exert anti-myeloma activity equal to that of V $\delta$ 2 T cells. *Conclusions.* We have shown for the first time that V $\delta$ 1 T cells are highly myeloma-reactive and have therefore established V $\delta$ 1  $\gamma\delta$  T cells as a potential candidate for a novel tumor immunotherapy.

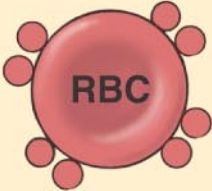
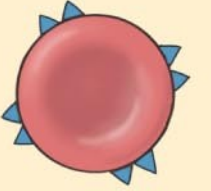
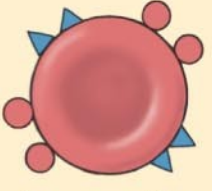



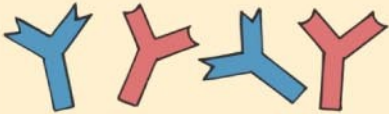
# SCT and CMV

- HCMV cytomegalovirus
- Common beta-herpes virus (HHV5)
- Primary infection followed by a latent infection
- Vigorous immune response, persistent suppression of viral replication
- CMV seropositivity associated with **immune senescence** of virus-specific CD4+ and CD8+ T cells (**Prof. Paul Moss, Graham Pawelec, Mark Wills**)
- Multiple strategies to evade the host immune system
- Immunocompetent vs immunocompromised host
  - Donor+ Recipient+
  - D+ R-
  - D- R+
  - D- R-

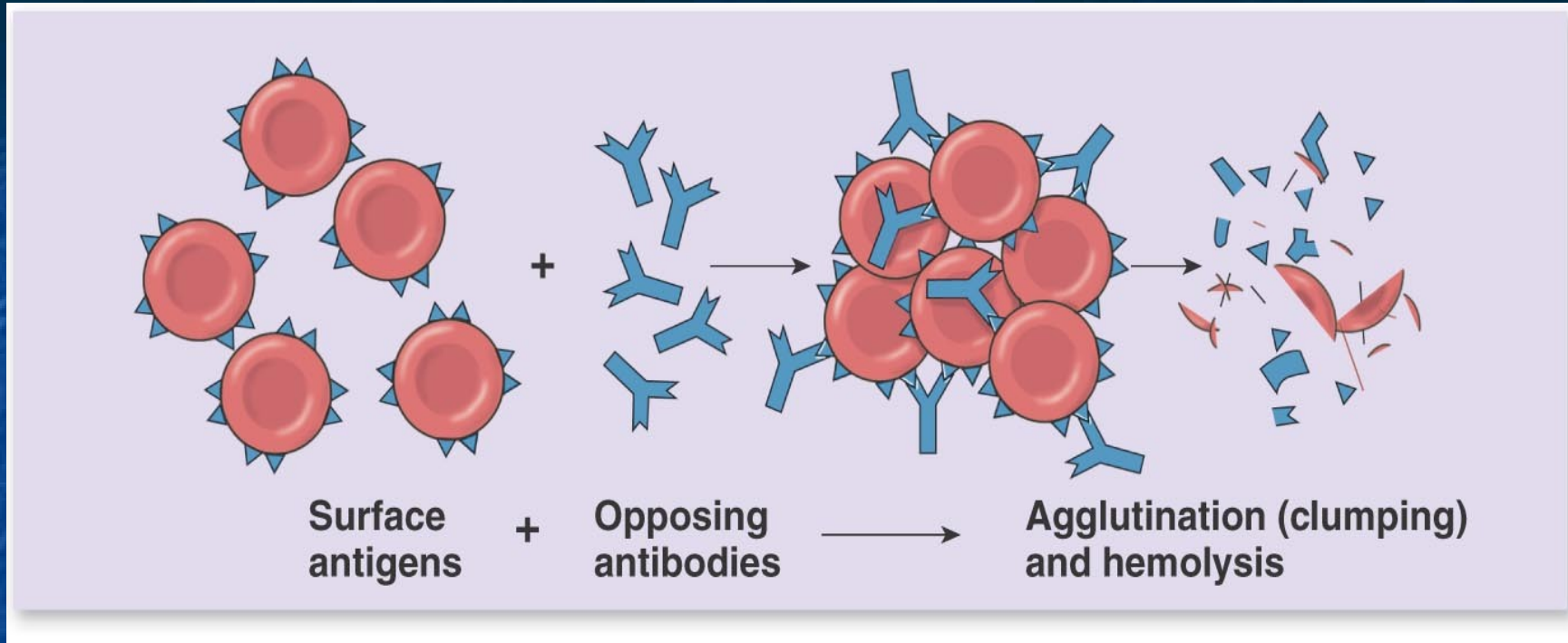
# Blood transfusion

- process of receiving blood intravenously
- to replace a lost blood component (red blood cells, plasma, platelets or clotting factors)
- donated blood processed/separated by centrifugation
- tested for infections (HIV 1, 2, HTLV 1, 2, Hep B, C , syphilis and CMV)
- stored in Blood Bank
- compatibility testing between D and R
- typing of recipient's blood determines the ABO blood groups and Rh status
- sample tested for any alloantibodies that may react with donor blood

# ABO blood groups

TYPE A	TYPE B	TYPE AB	TYPE O
 <p>RBC</p> <p>Surface antigen A</p>	 <p>Surface antigen B</p>	 <p>Surface antigens A and B</p>	 <p>Neither A nor B surface antigens</p>
 <p>Anti-B antibodies</p>	 <p>Anti-A antibodies</p>	<p>Neither anti-A nor anti-B antibodies</p>	 <p>Anti-A and anti-B antibodies</p>

(a)



- If a blood transfusion is given to a person who has antibodies to that type of blood, then the transfused blood will be attacked and destroyed (transfusion reaction)



# ABO blood group types

## ■ Europe:

- A 45%
- B 16%
- AB 6%
- O 33%

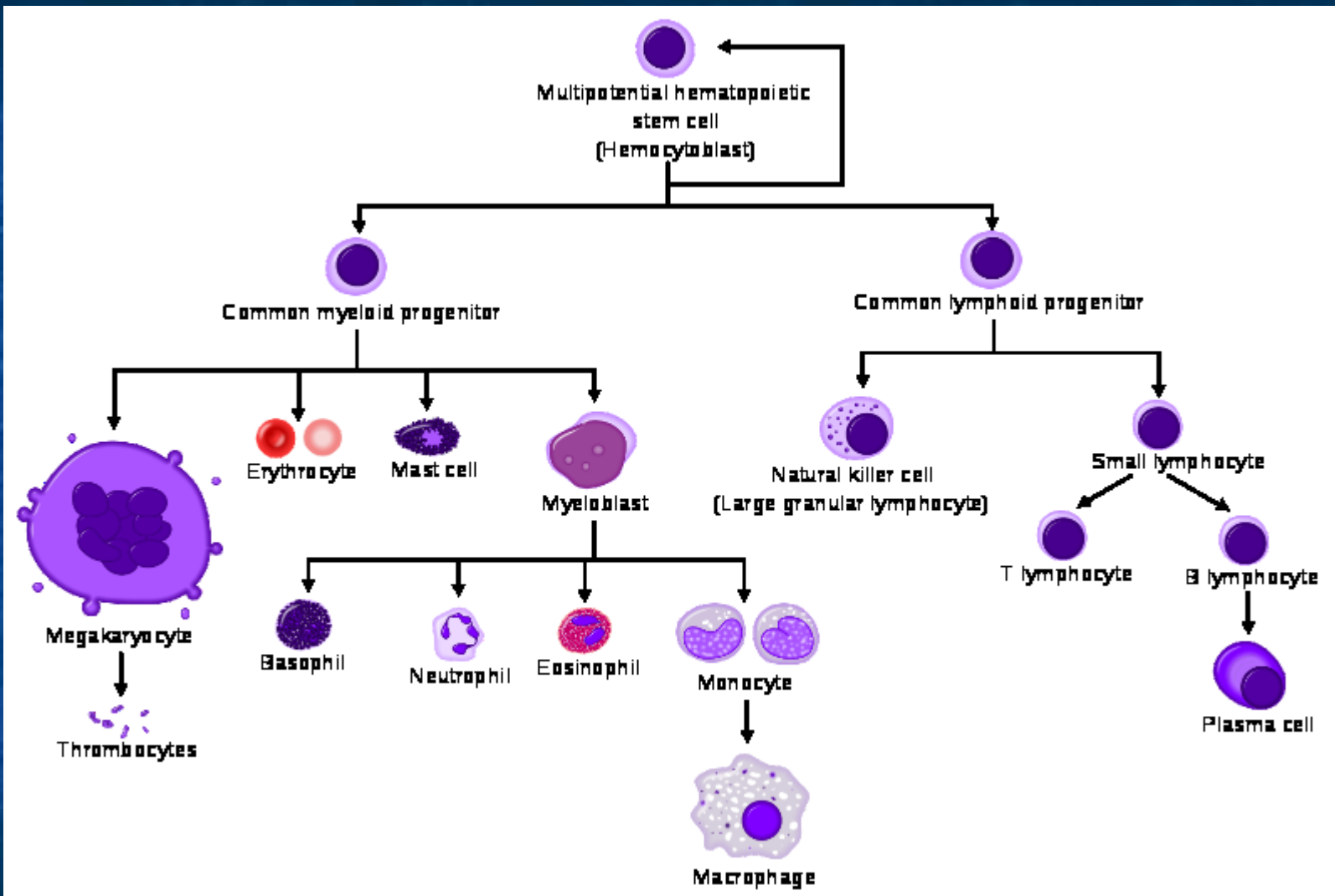
**TABLE 20.4 Differences in Blood Group Distribution**

Population	Percentage with Each Blood Type				
	O	A	B	AB	Rh+
<b>U. S. (average)</b>	46	40	10	4	85
Caucasian	45	40	11	4	85
African-American	49	27	20	4	95
Chinese	42	27	25	6	100
Japanese	31	39	21	10	100
Korean	32	28	30	10	100
Filipino	44	22	29	6	100
Hawaiian	46	46	5	3	100
<b>Native North American</b>	79	16	4	<1	100
<b>Native South American</b>	100	0	0	0	100
<b>Australian Aborigines</b>	44	56	0	0	100

# Rh blood group system

- consists of 50 defined blood-group antigens
- The commonly used terms *Rh factor*
  - *Rh positive* (85%)
  - *Rh negative* (15%) refer only to the *D antigen*
- We either have or don't have it on the surface of red cells
- Condition of hemolytic disease of the newborn
  - Incompatibility between mother and the fetus

# Haematological disorders



# Disorders of Erythrocytes

- **Polycythemia:** too many cells
- **Anaemia:** not enough cells

# Anaemia - symptoms

- Among over 400 types of anaemia
- Defined as a condition that develops when:
  - Decrease in the total number of red blood cells (RBC)
  - Decrease of the amount of haemoglobin and/or its reduced ability to carry oxygen
- Symptoms:
  - fatigue and tiredness, pain, shortness of breath, fast heartbeat, cold hands
  - bone deformities (found in thalassemia major)
  - leg ulcers (sickle cell anaemia)
  - enlarged spleen
  - in children - poor performance at school
  - in elderly, in patients

# Anaemia - causes

- **Blood loss**
  - Common in women, pregnancy
  - Acute: trauma and surgery
  - Chronic: many types of cancers (colon, bladder carcinomas), IBD patients
- **Decreased production of RBC - result of BM failure & differentiation of stem cells**
  - Pure red blood cell aplasia (PRCA)
  - Aplastic anaemia; along with exposure to chemicals, radiation, drugs, viral infection
  - Fanconi anaemia; 22x identified genes involved in DNA repair
- **Impaired/ faulty production of RBC and maturation of erythroblasts**
  - Deficiency of Vitamin B12; Pernicious anaemia
  - Iron deficiency anaemia; deficient heme synthesis
  - Severe type Myelophthisis; displacement of BM by malignant tumours or fibrosis
  - Myelodysplastic syndrome (MDS)
- **Increased destruction of RBC**

# Increased destruction of RBC classified as hemolytic anaemias

- **Hereditary spherocytosis**

- defect in RBC cell membrane caused by mutations in gene relating to proteins that allow RBC to change shape; then destroyed by the spleen

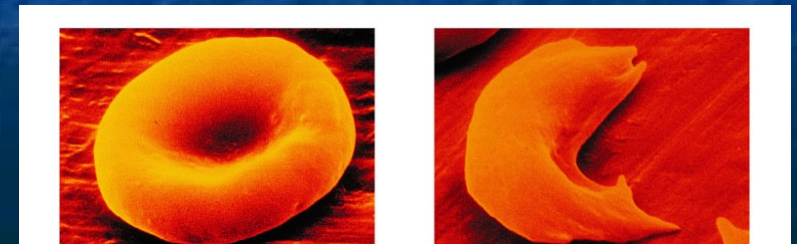
- **Antibody mediated**

- primarily by IgG attacking the RBCs, leaving their Fc exposed to monocytes and MΦ, destroyed as spherocytes in the spleen
- Warm autoimmune hemolytic anaemia (WAIHA) at 37°C
- Cold antibody induced anaemia (at 28-31°C)



# Disorders of Erythrocytes - Hemoglobinopathies

- are inherited single-gene disorders
- characterized by decreased and/or unstable haemoglobin
  - Thalassemia
    - usually results in underproduction of normal globin proteins often through mutations in regulatory genes
    - Beta; subtypes major (both beta globin genes missing) and intermedia
    - Alpha; subtypes Hb H and hydropsis fetalis
    - Minor; either alpha or beta globin gene missing
  - Sickle cell disease
    - Estimated that 7% of world's population (~420 million) are carriers
    - Inheritance of two abnormal B-globin gene (chr 11)
    - The gene defect is a SNP (single nucleotide polymorphism) where GAG changes to GTG and results in glutamic acid being substituted by valine (E6V)



# G6PD Deficiency

- Glucose-6-phosphate dehydrogenase deficiency
  - enzyme involved in the pentose phosphate pathway
  - important in red blood cell metabolism
- Perhaps most common, world-wide congenital abnormality
  - > 300 variants identified
  - X-linked inheritance
- Common G6PD deficient variants are associated with an acute intermittent hemolysis and anemia
- vast majority never symptomatic!
- Mediterranean and others: may hemolyze with fava beans

# Anaemia - diagnosis and treatment

## ■ Diagnosis

- Blood test, physical exam, symptoms and medical history
- Complete blood count (CBC) to determine the number, size and volume of Hb
- Blood iron level to indicate of body's iron stores
- Vitamin B12 levels
- Detection of rare types, RBC fragility, reticulocyte count, bilirubin

## ■ Treatment

- Iron and folic acid supplements
- Blood transfusion

# Disorders of Platelets

## ■ Thrombocytopenia

- normal platelet count ranges from 150,000 - 450,000 per  $\mu\text{L}$
- platelet count below 50,000 per  $\mu\text{L}$
- occasional bruising, nosebleeds, bleeding gums
- !! internal bleeding
- many causes: decreased production or increased destruction (SLE, HIV)
  - Vitamin B12 or folic acid deficiency
  - Leukaemia, MDS
  - Decreased production of thrombopoietin by the liver in liver failure
  - Bacterial, viral infections, sepsis
  - Hereditary: Fanconi anemia
- Treatment depending on the cause
  - Corticosteroids
  - Platelet transfusion

# Disease of the bone marrow

- Congenital defects
- Aplastic anemia
- Malignancies
  - Leukaemia
  - Lymphoma
  - Multiple myeloma

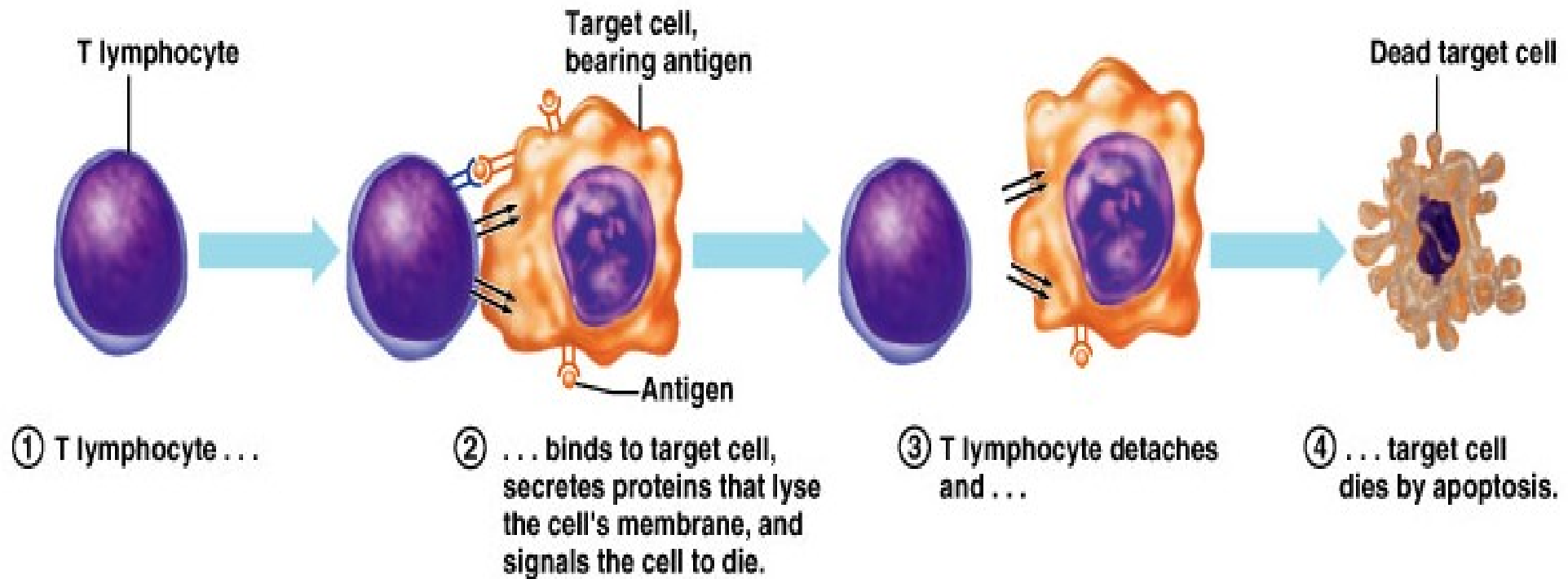
# Congenital defects

## ■ Dyskeratosis congenita (DKC)

- is a rare progressive congenital disorder resembling premature aging
- Essen. bone marrow failure syndrome
- DKC typically develop between ages 5-15 years
- is a result of one or more mutations in the long arm of the chr X **in the gene DKC1**
- Heiss NS, [Knight SW](#), Vulliamy TJ, et al." May 1998, *Nat. Genet.* **19** (1): 32–38

# Haematological Malignancies - Objectives

- Define the disease: acute vs chronic leukemia...
- Classify leukemia
- Understand the pathogenesis
  - Genetic alterations including translocations, mutations (leukaemogenesis)
- Understand the pathophysiology
- Able to list down the laboratory investigations required for diagnosis
- Understand the basic management of leukemia patients



(a)

Stress ligands  
shedding  
trogocytosis



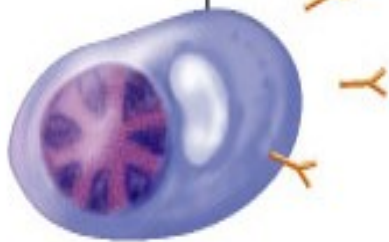
B lymphocyte



① B lymphocyte ...



Plasma cell



② ... gives rise to plasma cell,  
which secretes antibodies.

Antibodies



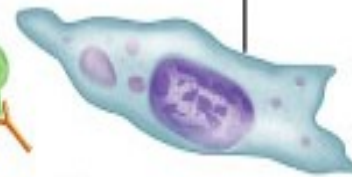
Bacterium



Surface antigen

③ Antibodies bind to  
antigens on bacteria,  
marking the bacteria  
for destruction.

Macrophage



④ Antibody-coated  
bacteria are avidly  
phagocytized.

(b)

# Leukaemia I.

- Definition:
- heterogenous group of malignant disorders which is characterised by uncontrolled clonal and accumulation of blasts cells in the bone marrow and body tissues
- Excessive production of WBC
- Often non fully differentiated cells called "blasts"
- WBC have abnormal function
  - Resistant to apoptosis
  - Excessive proliferation
  - Tumour microenvironment in the bone marrow
- Disruption of normal haematopoiesis in bone marrow

# Leukaemia II.

- Classification

- **Acute**

- Acute lymphoblastic leukemia (T-ALL & B-ALL)
    - Acute myeloid leukemia

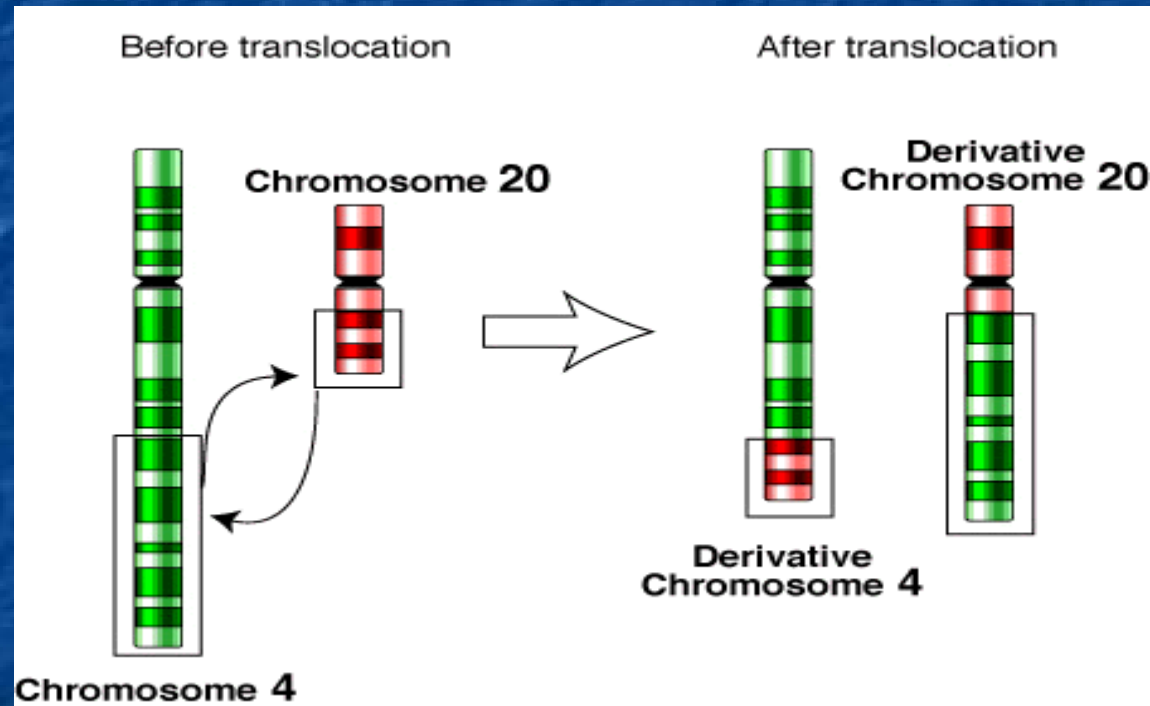
- **Chronic**

- Chronic myeloid leukemia
    - Chronic lymphocytic leukemia

# Leukaemogenesis

- Develops as a result of a genetic alteration within single cell in the bone marrow
  - Hereditary factors (Fanconi, Down sy)
  - Radiation, chemicals, drugs
  - Virus related
  - Oncogenes, tumour suppressor genes
  - Retrovirus mediated (HTLV-1, EBV)
  - Age related

# Leukaemia and chromosomal translocations



# Mechanisms of Translocation

- Ionising radiation can cause breakage of the phosphodiester backbone of both strands of DNA
- Double-strand breaks are very efficiently repaired
  - Potential loss of genetic material
  - Double-strand ends recognised as “foreign” DNA and destroyed
- If Double-strand breaks occur in two different chromosomes then possibility for incorrect repair taking place

# Frequent translocations

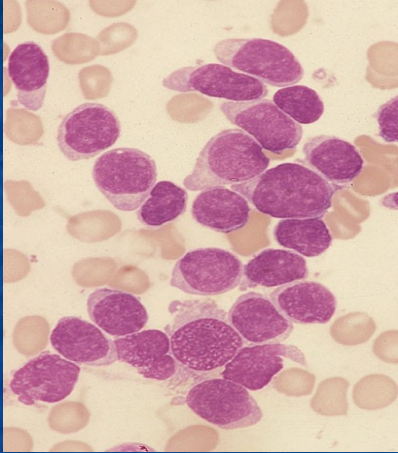
B-ALL	t(1;19)	5%
B-ALL (in children)	t(12;21)	22%
T-ALL	t(5;14)	20%
T-ALL	1p32 deletion	25%
AML	t(15;17)	13%
AML	t(8;21)	7%
CML	t(9;22)	99%

# Techniques used in Molecular Diagnosis

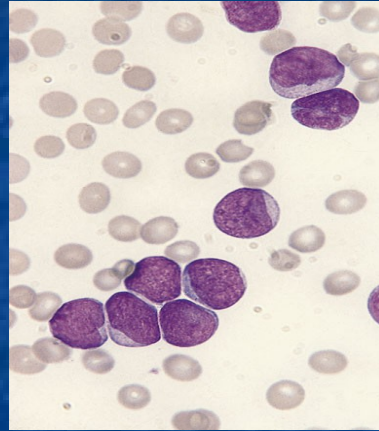
- Morphology
- Flow Cytometry
- PCR
- FISH



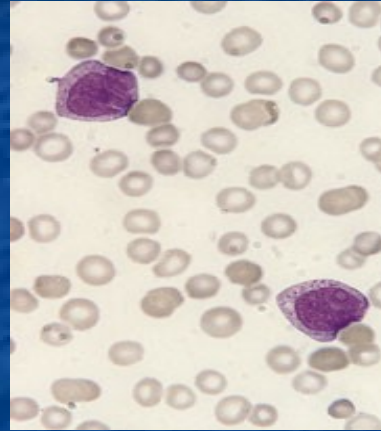
# AML morphology



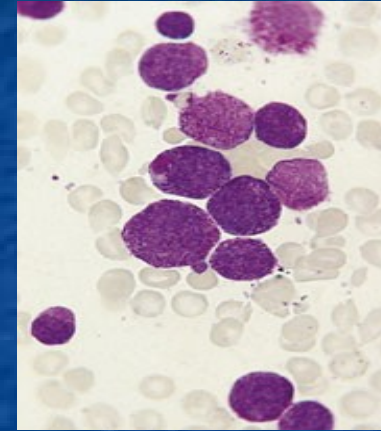
m0



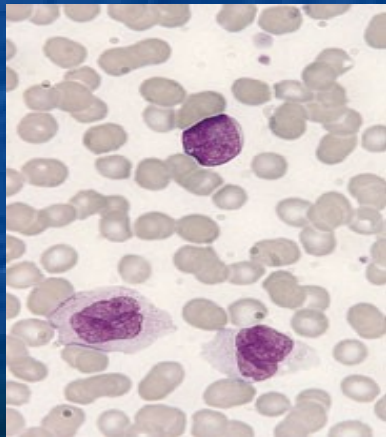
m1



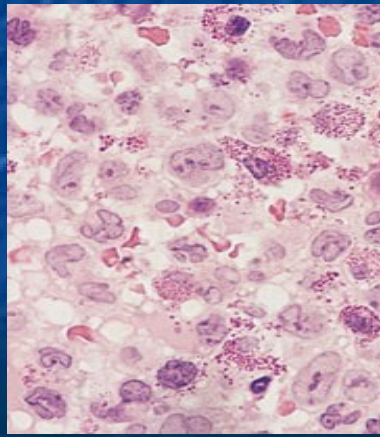
m2



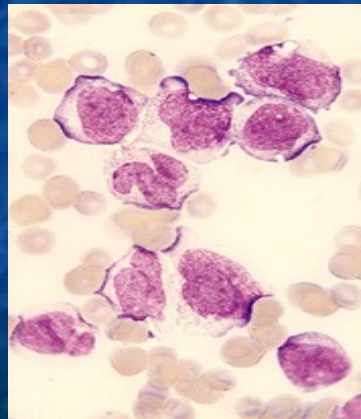
m3



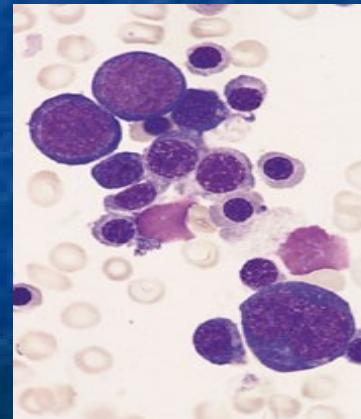
m4



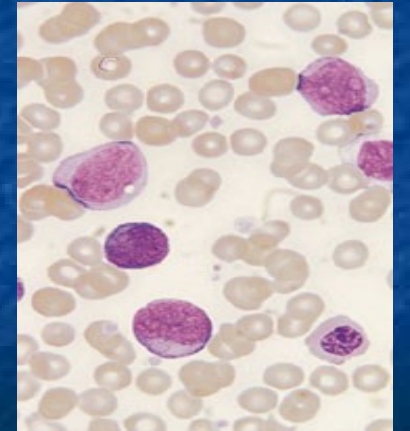
m4eo



m5



m6



m7

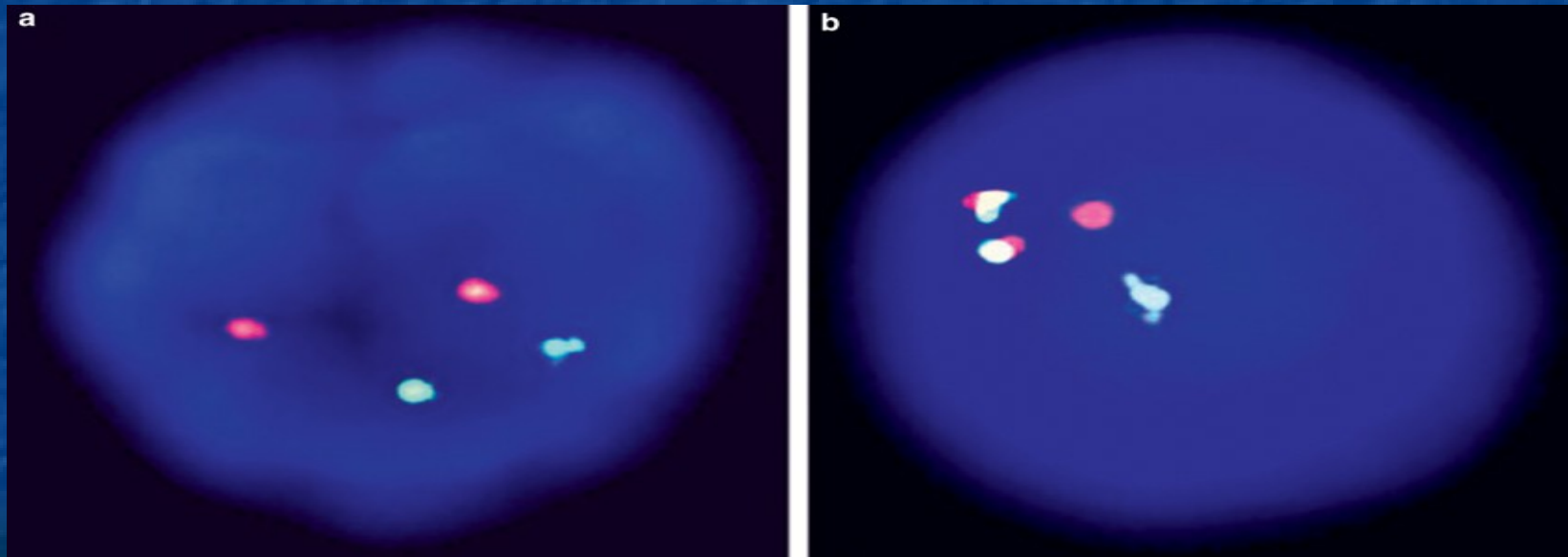
# FAB classification of AML

- M0 Undifferentiated blasts
- M1 AML without maturation
- M2 AML with maturation
- M3 Acute promyelocytic leukemia
- M4 Acute myelomonocytic leukemia
- M5 Acute monocytic leukemia
- M6 Acute erythroblastic leukemia
- M7 Acute megakaryoblastic leukemia

# Fluorescence *in situ* hybridization (FISH)

- Use of fluorescently-labeled DNA probes to hybridize onto metaphase spread of chromosomes
- Allows for the location of the probe on the chromosome to be identified

t(15;17)



Fluorescence *in situ* hybridization investigation of cutaneous lesions in acute promyelocytic leukemia  
Wrede et al. *Modern Pathology* (2005) **18**, 1569–1576.

# Acute Leukaemogenesis

**Oncogene can be activated by :**

- **chromosomal translocation**
  - **point mutations**
  - **inactivation**
- 
- In general, several genes have to be altered to effect neoplastic transformation

# Pathophysiology

- Acute leukaemia cause morbidity and mortality through:
  - **Deficiency in blood cell number and function**
  - **Invasion of vital organs**
  - **Systemic disturbances by metabolic imbalance**

# Acute Lymphoblastic Leukaemia

- Cancer of the blood affecting the white blood cell *LYMPHOCYTES*
- Commonest in the age 2-10 years
- Peak at 3-4 years.
- Incidence decreases with age, and a secondary rise after 40 years.
- In children - most common malignant disease
- 85% of childhood leukaemia

# Acute Myeloid Leukaemia

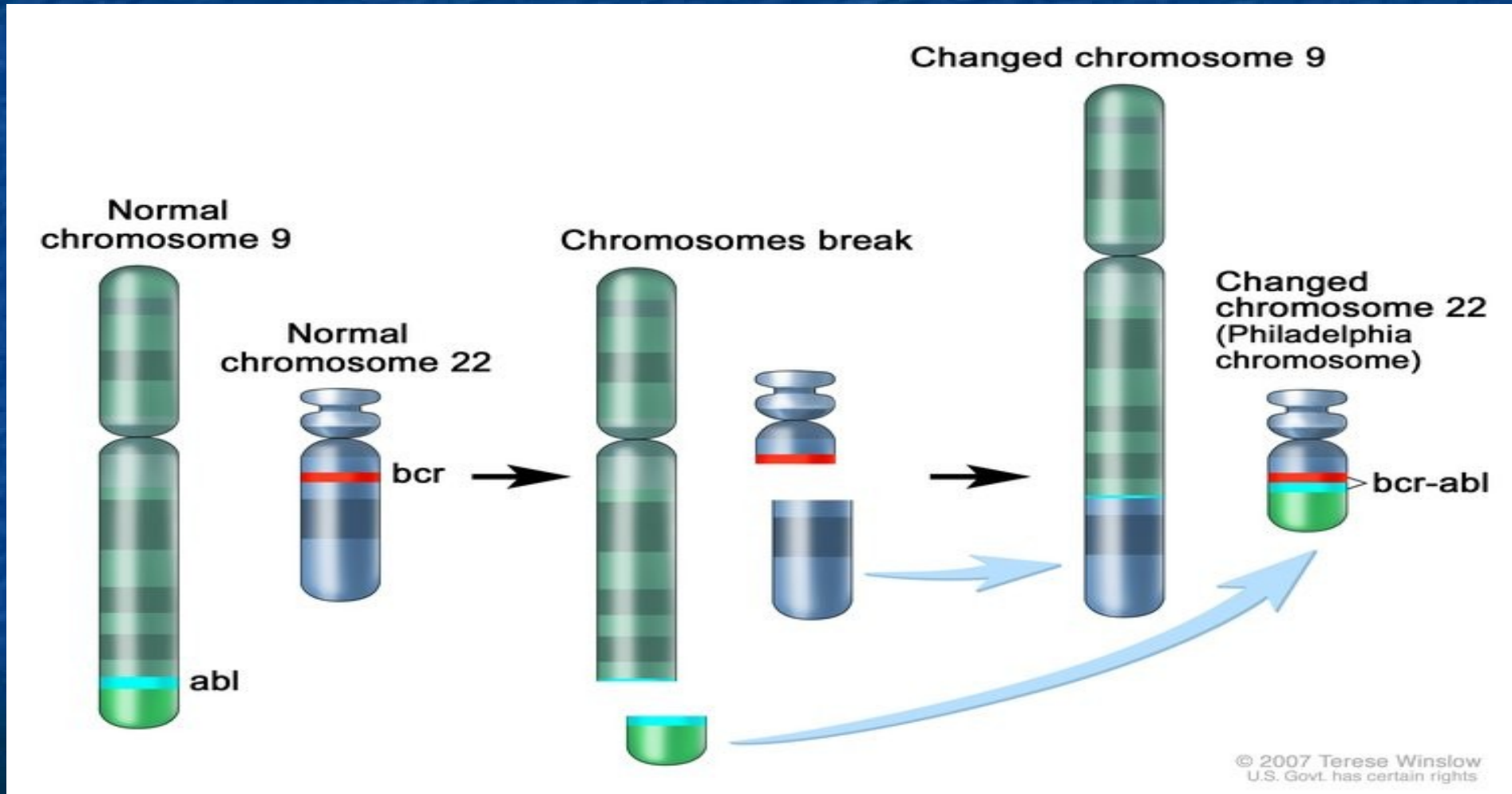
- Arise from the malignant transformation of a myeloid precursor
- Rare in childhood (10%-15%)
- The incidence increases with age
- 80% in adults

# Molecular biology of CML

- 90% of patients have Philadelphia Chromosome (Ph)
- t(9;22) balanced translocation
- disruption of the ABL (Chr 9) and BCR (Chr 22) genes
- formation of two hybrid genes
  - 5'BCR/3'ABL
  - 5'ABL/3'BCR
- Only the BCR/ABL hybrid gene is active
  - BCR/ABL mRNA
  - p210 'fusion' protein



# t(9;22) translocation in CML



# Why is p210 (BCR/ABL) protein oncogenic?

- ABL
  - protein tyrosine kinase
  - interacts with the adaptor protein CRKL
- BCR
  - interacts with the adaptor protein Grb2
- CRKL and GRB2 activates the RAS growth stimulation signalling pathway
  - Inactivation of pro-apoptotic protein (BAD)
  - Up-regulation of the anti-apoptotic protein (BCL<sub>XL</sub>)
- p210 is a constitutively active tyrosine kinase resulting in the permanent activation of the RAS pathway

# New CML Treatment

- Design compounds that specifically target the p210 protein
- p210 is CML specific
- imatinib (Gleevec, Glivec, STI571)
- specifically inhibits the ABL kinase
  - imatinib inhibits the growth of CML cells in culture
  - Progression-free survival at 24 months is 87%
- Prof John Goldman Hammersmith Hospital London
- Prof John Barret NIH Washington
- Prof Francois Mahon Bordeaux
- Prof Ráčil, Prof Mayer FN Brno

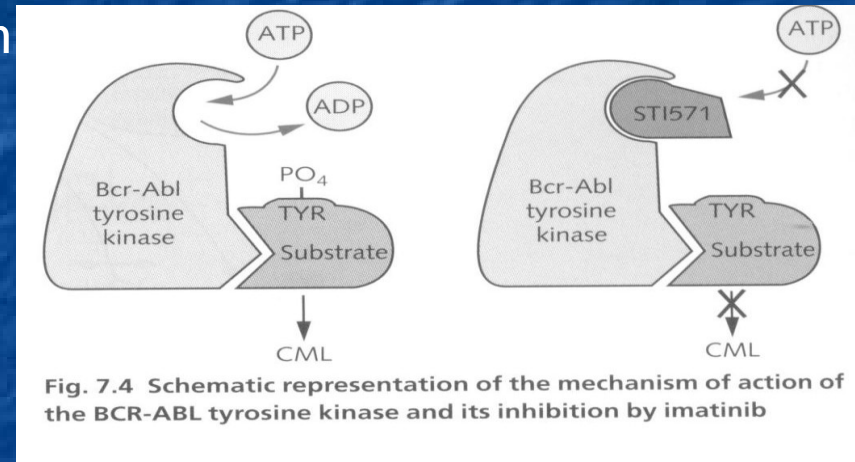


Fig. 7.4 Schematic representation of the mechanism of action of the BCR-ABL tyrosine kinase and its inhibition by imatinib

*Molecular Haematology Provan & Gribben*

# Chronic lymphocytic leukaemia

- Most common leukaemia in the Western countries
  - lymphocytosis of  $> 5000$  cells/ $\mu$ l for
  - $> 3$  months
- Flow cytometry of peripheral blood (phenotype CD19, CD5, CD23)
- Bone marrow biopsy
- Staging according to Rai (I-IV)
- Mutated IgVH
- Del11q (ATM)
- Del17p
- Del13q (RB1)
- +12
- TP53
- Prof Michael Doubek, IHOK, FN Brno

# Multiple Myeloma

- B cell malignancy of plasma cells CD38+CD138+ in the bone marrow
- Pre-malignant stage:
  - MGUS – monoclonal gammopathy of undetermined significance
  - Progression of 1% per annum
- Bone marrow biopsy
- Therapy (IMiDS)
- Prof. Roman Hájek FN Ostrava

# Questions